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SINGLE AND BLENDED MAIZE VOLATILES AS ATTRACTANTS FOR DIABROTICITE CORN ROOTWORM BEETLES[1]

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Abstract—Synthetic maize volatiles and analogs dispensed singly and blended were tested for attractiveness to western (WCR, *Diabrotica virgifera virgifera*) and northern corn rootworm beetles (NCR, *D. barberi*) in maize fields. Newly identified attractants included *syn*-benzaldoxime, especially for NCR, and β-caryophyllene for WCR females. ($\pm$)-Linalool was more effective than was ($-$)-linalool. Myrcene, (+)-β-pinene, and ($-$)-β-pinene were unattractive. Adding methyl salicylate to ($\pm$)-linalool, (+)-α-terpineol, or β-ionone appeared to synergistically increase capture of WCR females, but dispensing the terpenes in binary blends did not. Dose–response data for methyl salicylate, ($\pm$)-linalool, and a blend of both compounds confirmed the synergy. β-Caryophyllene, but not ($-$)-α-pinene, added to the latter blend produced a further synergistic increase in WCR female capture that did not vary with sesquiterpene dose from 1.0 to 100 mg. Indole addition to the same blend caused an increase in WCR female captures indicative of synergy, assuming that each did not individually lure different segments of the WCR female population. The green leaf volatiles *(Z)*-3-hexenyl acetate and (*Z*)-3-hexen-1-ol were unattractive alone and had no influence on efficacy of traps baited with 3.3 mg each of ($\pm$)-linalool, methyl salicylate, and β-caryophyllene. The latter mixture captured about half as many WCR females as did 10 mg of 4-methoxycinnamaldehyde, a potent WCR attractant standard. Substituting β-ionone for ($\pm$)-linalool yielded a ternary blend that captured more beetles than did the aldehyde and was unaffected by aldehyde addition. Olive oil, which has been used to sustain attractant volatilization, did not affect captures. The results show that the blending of maize volatiles has the

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potential to greatly improve efficacy of lures having promising applications in corn rootworm population management.

Key Words—Coleoptera, Chrysomelidae, *Diabrotica virgifera virgifera, Diabrotica barberi, Zea mays,* maize volatiles, host plant attractant, kairomone, synergy.

INTRODUCTION

Diabroticite rootworms (Coleoptera: Chrysomelidae) are major pests of maize, *Zea mays* L., in the United States, where most damage results from western (WCR) and northern (NCR) corn rootworms, *Diabrotica virgifera virgifera* LeConte and *D. barberi* Smith and Lawrence, respectively. The WCR was also recently introduced into Europe, where it is spreading and attaining pest status (Enserink, 1999). Principal rootworm management options are crop rotation and chemical insecticides. The latter account for nearly a fifth of the insecticides applied to U.S. field crops (Delvo, 1993). New management tools are needed to address pesticide-related environmental concerns and the emergence of corn rootworm populations adapted to resist insecticides (Meinke et al., 1998) or survive in maize rotated with another crop (Krysan, 1993; Stewart et al., 1995; Onstad et al., 1999). One promising approach is the exploitation of insect behavior-modifying chemicals. These include cucurbitacin feeding stimulants, currently under evaluation in area wide corn rootworm trials, and host plant kairomones or their analogs with odors attractive to adult diabroticite beetles (Levine and Oloumi-Sadeghi, 1991; Sutter and Lance, 1991; Metcalf and Lampman, 1997).

Because most crop damage from corn rootworms results from larval feeding, kairomones attracting adults could be most effectively used to monitor the potential for damaging larval populations in subsequent growing seasons or to interfere with reproduction by mass trapping or annihilation techniques. Unlike diabroticite sex pheromones, the kairomonal attractants, which apparently influence host seeking and selection, usually trap at least as many females as males, enhancing their potential as rootworm management tools (Quiring and Timmins, 1990).

Attraction of diabroticite beetles to host plant odors has been studied in considerable detail. Phytochemicals from Cucurbitaceae, the hypothesized ancestral hosts of diabroticite corn rootworms (Metcalf and Metcalf, 1992), have been most extensively studied (Metcalf and Lampman, 1997; Cossé and Baker, 1999); however, synthetic maize volatiles were examined in recent field trials (Hammack, 1996, 1997). Existing research stresses phenylpropanoids from cucurbits and terpenoids from maize, but diabroticite attractants show considerable chemical heterogeneity, and some, such as β-ionone, indole, 2-phenyl-1-ethanol, and phenylacetaldehyde, occur in both maize and squash blossoms (Flath et al., 1978; Buttery et al., 1980; Andersen, 1987; Andersen and Metcalf, 1987; Turlings et al., 1993). The green leaf volatiles (GLVs), aliphatic six-carbon primary alcohols, aldehydes, and acetates that are ubiquitously distributed in the plant kingdom, also occur in both host groups, but have received little attention as corn rootworm attractants.

The chemical diversity of diabroticite attractants identified to date and their widespread distribution among plant families increase the likelihood that odorant blends are important for specificity and strength of adult host-finding responses. Although existing studies emphasize individual compounds, several blends of cucurbit blossom volatiles are known to produce a synergistic increase in capture of WCR, NCR, or southern corn rootworm, *D. undecimpunctata howardi* Barber (SCR). These blends include equal-weight mixtures of veratrol, indole, and phenylacetaldehyde (VIP) for SCR and 1,2,4-trimethoxybenzene, indole, and (*E*)-cinnamaldehyde (TIC) for all three species (Lampman and Metcalf, 1987). Indole was recently identified as a key synergist in both VIP and TIC mixtures and in combination with 4-methoxycinnamaldehyde, the most potent single-component attractant available for WCR and a possible contributor to cucurbit blossom aroma (Metcalf et al., 1995; Metcalf and Lampman, 1997). More limited evidence that lure efficacy can be increased by blending attractants has also been reported (Hammack, 1996; Metcalf and Lampman, 1997; Petroski and Hammack, 1998), including evidence for synergy between maize terpenoids.

Improved efficacy of corn rootworm kairomonal lures is desirable, at least for mass trapping and annihilation applications. This is because the kairomonal lures generally function least well, if at all, during maize silking, and intervention with these techniques would work best during or shortly after silking when females have emerged but not yet oviposited to any extent (Lance, 1993; Hesler et al., 1994; Hammack and Hesler, 1995).

The present study tested primarily maize terpenoids, GLVs, and blends of these chemicals for attractiveness to WCR and NCR beetles. The main goal was to assess the blending of individual attractants as a means of improving lure efficacy, emphasizing inclusion of maize headspace volatiles in test blends. Attention to blends also stimulated evaluation of the need for a previously used olive oil extender (Hammack, 1997) because of the possible existence of behaviorally active oil volatiles and the greater potential for interaction of any such volatiles with blended test components.

METHODS AND MATERIALS

Odorants. The structure and purity of the test compounds are shown in Figure 1. They were purchased from Fluka Chemical Co., Ronkonkoma, New York {(−)-linalool, β-caryophyllene [(-)-*trans*-caryophyllene], (+)-α-terpineol, (+)- and (−)-β-pinene}; Sigma, St. Louis, Missouri [(*Z*)-3-hexenyl acetate];

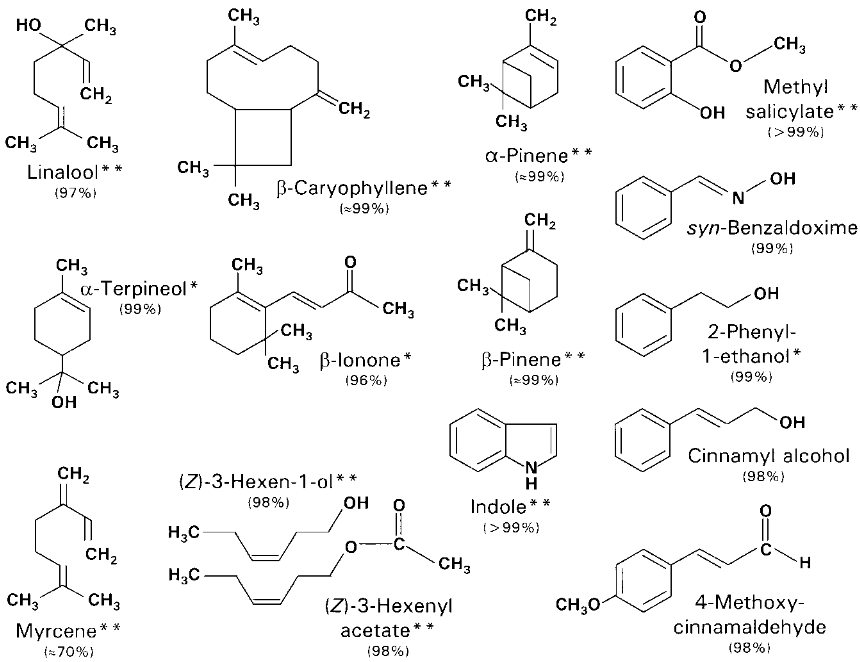

FIG. 1. Structure and purity (in parentheses) of test compounds. Single asterisks indicate maize volatiles (Flath et al., 1978). Double asterisks indicate those maize volatiles found in headspace analyses (see text for references).

Schweizerhall Inc., South Plainfield, New Jersey (4-methoxycinnamaldehyde); or Aldrich, Milwaukee, Wisconsin (all others).

Experiments. Ten field experiments were conducted between 1996 and 1999. One 1996 test examined the effect of omitting the olive oil extender on WCR and NCR responses to 2-phenyl-1-ethanol and to unbaited controls. This alcohol was used because it lures beetles of both species (Petroski and Hammack, 1998).

A second 1996 test screened single compounds for attractiveness. Four terpenoids (β-caryophyllene, linalool, myrcene, and β-pinene) were assayed because of their presence in maize headspace (Buttery and Ling, 1984; Light et al., 1993; Turlings et al., 1993; Takabayashi et al., 1995). *syn*-Benzaldoxime was tested because of its structural similarity with 2-phenyl-1-ethanol, a lure shared by maize and cucurbits, and with 2-phenyl-1-ethylamine, an effective NCR attractant not reported from host plants (Metcalf and Lampman, 1991; Petroski and Hammack, 1998). Cinnamyl alcohol was included as a reference standard for NCR, for lack of a maize headspace volatile highly attractive to this species. (±)-Linalool, previously shown to lure primarily WCR (Hammack, 1997), served as a standard for WCR and was compared with (−)-linalool.

The remaining 1996 test screened for synergy among four maize volatiles known to individually attract primarily WCR: (±)-linalool, (+)-α-terpineol, β-ionone, and methyl salicylate (Lampman and Metcalf, 1988; Hammack, 1996, 1997). Screening was done by comparing responses to one-component, binary, and quaternary blends. The compounds were mixed in equal weights for convenience.

One of two 1997 tests yielded dose–response data for (±)-linalool, methyl salicylate, and an equal weight blend of both compounds to confirm synergy between them. Linalool was used for confirmation, rather than α-terpineol or β-ionone, because it is the only one of the three terpenes so far reported in maize headspace analyses (Buttery and Ling, 1984; Light et al., 1993; Takabayashi et al., 1995; Turlings et al., 1993).

The other 1997 test appraised synergy upon β-caryophyllene addition to the blend of (±)-linalool and methyl salicylate. This test was repeated twice, except that β-caryophyllene was replaced with one of two maize headspace volatiles: the attractant indole in 1998 and (−)-α-pinene in 1999. (−)-α-Pinene earlier showed evidence of slight attractiveness to WCR in one of two tests when dispensed by itself (Hammack, 1997).

The effect on beetle captures of altering the ratio of blend components was examined in 1998 by varying from 1 to 100 mg the amount of β-caryophyllene dispensed with 10 mg each of (±)-linalool and methyl salicylate. Another 1998 test evaluated attractiveness of two GLVs released by maize, (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate (Buttery and Ling, 1984; Light et al., 1993; Takabayashi et al., 1995; Turlings et al., 1993). The GLVs were dispensed individually and blended with 3.3 mg each of (±)-linalool, methyl salicylate, and β-caryophyllene. A final 1998 test aimed to enhance captures by replacing (±)-linalool with β-ionone in the ternary blend and to compare efficacy of the maize blends with that of 4-methoxycinnamaldehyde. The latter aldehyde was also dispensed in combination with the maize volatiles.

Bioassays. Cotton dental rolls (1 cm diam. 3.8 cm long) were used in all of the experiments to dispense the test odorants. Each roll was also treated in 1996 with 2.0 ml of olive oil in hexane (1:4 by volume) (Hammack, 1997), except for the test which compared attractiveness of 2-phenyl-1-ethanol dispensed with and without the extender. No extender was used after 1996. Odorant-treated dental rolls were attached for bioassay to yellow Pherocon AM (Trécé, Inc., Salinas, California) sticky traps as the traps were placed in grower maize fields in Brookings County, South Dakota. When multiple test compounds were to be dispensed from the same trap, each was applied to a half dental roll (1.9 cm long) that was separately affixed to the trap. The number of dental rolls and solvent volumes per trap (oil and hexane in 1996, acetone used to dissolve indole and 4-methoxycinnamaldehyde in 1998) were held constant within experiments. Control traps were identical to the treated ones, except that no test odorant(s) was applied to the dental roll(s) affixed to the controls. Odorants were considered attractive if they captured significantly more beetles than did the control.

The experiments were all laid out in a randomized complete block design. Each consisted of eight blocks, except for six blocks in two 1996 tests: the test that examined effects of extender and the one that screened individual compounds for attractiveness. Traps were positioned at least 30 m from one another at ear height on maize plants, and crop phenology was determined during testing, as previously detailed (Hammack, 1996, 1997). Traps were left in the field for 48 hr, except for 96 hr when 2-phenyl-1-ethanol was dispensed with and without extender and 24 hr in the $(-)$-α-pinene test (for convenience). The species and sex of captured beetles were recorded once the traps were returned to the laboratory.

Odorant Interactions. Interactions were evaluated using dose–response data and ratios of interaction (ROI), where $ROI = [(A + B) + \text{Control}]/[(A) + (B)]$, A is the capture on a trap baited with odorant A, B is the capture on a trap baited with odorant B or blend B, and $A + B$ is the capture on a trap baited with both A and B (Hammack, 1996). Odorants were deemed to interact synergistically when the response to a blend exceeded that predicted from their individual dose–response curves (Barenbaum, 1989) and, in addition, the ROI value was greater than one (Hammack, 1996). If the first criterion was met but the $\text{ROI} = 1$, then synergy could still be occurring if the individual odorants were similarly affecting the same segment of the test population. Although such similar action was likely, it was not unequivocally proven in these field experiments.

Statistical Analyses. The SAS Institute (1989) statistical package was used for data analysis. To determine whether ROI values differed from 1, Student's t test for paired samples or Wilcoxon's signed rank test (S statistic) was used to test the null hypothesis of no difference between ROI numerator and denominator (PROC Univariate). Wilcoxon's test was used instead of the t test if the Shapiro-Wilk test for normality approached significance at $P \leq 0.1$ (PROC Univariate). Otherwise, data from each experiment were transformed $[\ln(x + 1)]$ to ensure homogeneity of variances and then examined by analysis of variance (PROC ANOVA). The Student-Newman-Keuls option was used to separate means after a significant ANOVA. The tables and figures show untransformed data. $P \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Extender. Dose significantly influenced capture of both WCR and NCR on traps baited with 0–30 mg of 2-phenyl-1-ethanol, although WCR males were unaffected (Table 1). In contrast, treatment with the oil extender had no effect on captures of either sex or species nor did it interact with dose to alter captures (Table 1).

TABLE 1. CAPTURE OF CORN ROOTWORM ADULTS ON TRAPS BAITED WITH 2-PHENYL-1-ETHANOL ON DISPENSERS WITH AND WITHOUT OLIVE OIL[a]

		Capture (mean ± SE)			
		Western corn rootworm		Northern corn rootworm	
2-Phenyl-1-ethanol dose (mg/trap)	Oil	Female	Male	Female	Male
30	yes	87.8 ± 17.6	36.3 ± 3.9	97.0 ± 17.6	61.0 ± 9.7
30	no	91.7 ± 17.1	46.7 ± 7.2	85.3 ± 11.4	57.2 ± 6.1
3	yes	36.5 ± 5.2	43.8 ± 4.9	28.7 ± 5.9	46.2 ± 9.6
3	no	45.5 ± 7.6	51.7 ± 7.4	37.5 ± 5.1	55.5 ± 12.1
0	yes	12.8 ± 2.1	51.0 ± 10.3	4.0 ± 1.7	34.7 ± 6.5
0	no	14.8 ± 3.1	48.8 ± 8.8	5.8 ± 0.8	39.8 ± 2.8
F statistics[b]					
Oil ($df = 1, 25$)		0.36 NS	0.94 NS	2.79 NS	0.73 NS
2-Phenyl-1-ethanol dose ($df = 2, 25$)		48.90**	0.82 NS	114.4**	3.74*
Oil × dose ($df = 2, 25$)		0.14 NS	0.41 NS	1.45 NS	0.28 NS

[a] Test conducted August 30–September 3, 1996 ($N = 6$). Mean beetle count per plant ± SE on September 3 was 1.7 ± 0.3 WCR and 1.0 ± 0.2 NCR. Corn was in the dough stage (R4).
[b] Asterisks denote statistical significance at $^{*}P < 0.05$ or $^{**}P < 0.0001$. NS denotes $P > 0.05$.

Many extenders including olive and mineral oils have been used to sustain volatilization of candidate attractants from lure dispensers in field trials (Lampman and Metcalf, 1987; Petroski and Hammack, 1998; Cossé and Baker, 1999), although some, like olive oil, could release behaviorally active volatiles. The present study detected no effect of olive oil, either by itself or in combination with 2-phenyl-1-ethanol, on WCR or NCR captures. However, omission of the oil was still deemed desirable, at least in these short-term tests, because it precluded interaction with test compounds other than the alcohol and will facilitate future gravimetric measurement of attractant release rates.

Screening of Individual Terpenoids/Benzaldoxime. The screening of individual compounds (100 mg/trap) showed *syn*-benzaldoxime attractive to WCR females and to NCR of both sexes, while β-caryophyllene attracted only WCR females (Table 2). *syn*-Benzaldoxime captured more NCR than did the cinnamyl alcohol standard, considered a strong NCR attractant (Metcalf and Lampman, 1991), and as many WCR females as did (±)-linalool. β-Caryophyllene, in contrast, lured fewer WCR females than did the (±)-linalool standard. (−)-Linalool also proved less attractive than the racemic standard, especially to WCR but also to NCR females. Neither β-pinene stereoisomer nor myrcene was attractive (Table 2).

This is the first demonstration that syn-benzaldoxime and β-caryophyllene attract corn rootworm adults. Activity of the oxime may derive from a structural similarity with 2-phenyl-1-ethanol or 2-phenyl-1-ethylamine lures, or perhaps

TABLE 2. CAPTURE OF CORN ROOTWORM ADULTS ON TRAPS BAITED WITH CANDIDATE ATTRACTANTS[a]

Candidate/standard attractant (100 mg/compound)	Capture (mean ± SE)			
	Western corn rootworm		Northern corn rootworm	
	Female	Male	Female	Male
syn-Benzaldoxime	277.3 ± 17.4a	17.5 ± 2.6	410.7 ± 33.5a	144.5 ± 11.1a
β-Caryophyllene	65.7 ± 9.7b	13.0 ± 2.3	3.2 ± 1.6ef	11.7 ± 2.0d
(−)-Linalool	70.5 ± 8.0b	13.5 ± 2.0	10.3 ± 2.7d	20.3 ± 3.6c
(+)-β-Pinene	13.8 ± 1.9c	9.0 ± 1.4	1.0 ± 0.5f	9.7 ± 0.6d
(−)-β-Pinene	12.7 ± 2.0c	10.3 ± 0.7	2.2 ± 0.5ef	8.5 ± 2.0d
Myrcene	9.0 ± 2.0c	11.7 ± 3.1	1.7 ± 0.2ef	8.7 ± 0.9d
None (control)	15.2 ± 2.7c	13.0 ± 1.9	2.7 ± 0.3e	12.0 ± 2.0d
(±)-Linalool (WCR standard)	270.5 ± 39.0a	20.2 ± 3.2	20.3 ± 3.2c	22.3 ± 4.5c
Cinnamyl alcohol (NCR standard)	88.5 ± 19.2b	17.3 ± 1.9	180.3 ± 25.9b	93.3 ± 10.1b
F statistic[b]	63.56**	2.11 NS	109.58**	61.24**

[a] Test conducted September 9–11, 1996 ($N = 6$). Mean beetle count per corn plant ± SE on September 11 was 1.2 ± 0.2 WCR and 1.1 ± 0.2 NCR. Corn was in the dent stage (R5).
[b] Asterisks denote statistical significance at $P < 0.0001$, NS denotes $P > 0.05$ ($df = 8, 40$). Means within a column followed by the same letter do not differ by Student-Neuman-Keuls test, $P > 0.05$.

with phenylacetaldoxime, a common floral volatile (Kaiser, 1991). Oximes have not previously been reported as corn rootworm attractants and merit further study given the magnitude of WCR and especially NCR captures reported here.

β-Caryophyllene is released by a wide assortment of flowering plants (Knudsen et al., 1993; Borg-Karlson et al., 1994) and attracts insects other than WCR, including boll weevils (Minyard et al., 1969) and green lacewings (Flint et al., 1979). Like several other corn rootworm attractants, such as linalool and indole, β-caryophyllene is among volatiles released by maize in response to insect feeding (Turlings et al., 1993; Takabayashi et al., 1995). Its hydrocarbon structure renders β-caryophyllene an atypical attractant for diabroticite beetles. Several other maize terpenoid hydrocarbons, namely myrcene, β-pinene, and limonene (Light et al., 1993; Takabayashi et al., 1995), were tested here or earlier (Hammack, 1996), but failed to attract either WCR or NCR. (−)-α-Pinene, but not (+)-α-pinene, did attract WCR females in one of two earlier tests, although that response was only just detectable (Hammack, 1997) and not reproducible in the current study.

Unlike benzaldoxime, β-caryophyllene was a more effective WCR than NCR lure. Despite much overlap in the array of host compounds and analogs attractive to NCR and WCR beetles, response patterns of the two species are generally distinct (Hammack, 1996, 1997; Metcalf and Lampman, 1997; Petroski and Hammack, 1998). Distinct response patterns might be expected, as WCR and NCR likely evolved their association with maize independently of one another and differ as adults in their reliance on maize for food (Branson and Krysan, 1981). Metcalf et al. (1998) considered the greater reliance of WCR on maize atypical of diabroticite adults, which as a group tend to feed on pollen from a variety of grasses and forbs, perhaps in response by some *Diabrotica* species to widely distributed floral volatiles shared with ancestral Cucurbitaceae.

The weaker response of both species to (−)- than to (±)-linalool implies that(+)-linalool, or a blend containing this enantiomer, is the more effective lure. The (+)-isomer was not, however, available for testing. Chirality is known to be important to WCR reacting to α-terpineol (Hammack, 1996), as well as to female cabbage looper moths and solitary bees, which both preferentially respond to (*S*)-(+)-linalool acting as a pheromone (Heath et al., 1992; Borg-Karlson et al., 1996).

Traps baited with effective lures usually attracted more females than males within each species, and the disparity between the sexes tended to be greater for WCR than NCR. These results agree with those of previous studies, where trap catch was examined by sex (see Hammack, 1997 for references and further discussion), except that NCR females do not always outnumber NCR males. Assuming attractant involvement in host seeking/selection, higher female captures likely relate to nutritional demands of oogenesis, which could be more stringent in the more fecund WCR than NCR (Naranjo and Sawyer, 1987). Captures would also reflect sex ratios in maize fields at testing. Female to male ratios could tend to rise in late season with earlier mortality of males due to protandry (Quiring and Timmins, 1990) or, in the case of NCR, which are more apt than WCR to move out of maize to feed on a variety of flowering forbs, with the migration of females back into maize to oviposit (Branson and Krysan, 1981).

Terpenoid and Methyl Salicylate Blends. When methyl salicylate and the three terpenes were assayed at 30 mg per component per trap, each binary blend captured significantly more WCR females than did its individual constituents only when the blend contained methyl salicylate (Figure 2). A significant increase occurred with mixing when methyl salicylate was added to (±)-linalool, (+)-α-terpineol, or β-ionone, but not when blends contained any two of the three terpenes. Mixing all four chemicals failed to increase the capture of WCR females beyond that achieved by mixing only methyl salicylate and β-ionone. The latter two were the least and most active, respectively, of the four single compounds (Figure 2). The other two, (±)-linalool and (+)-α-terpineol, showed an intermediate attractiveness. WCR males demonstrated significant, albeit weak, olfactory attraction only when treatments included β-ionone; however, none of the β-ionone blends was significantly better than β-ionone by itself (Figure 2). Only treatments containing (+)-α-terpineol or (±)-linalool attracted NCR in numbers significantly higher than control, although mean captures per trap failed to exceed 25 and showed no tendency to increase when chemicals were blended (data not shown).

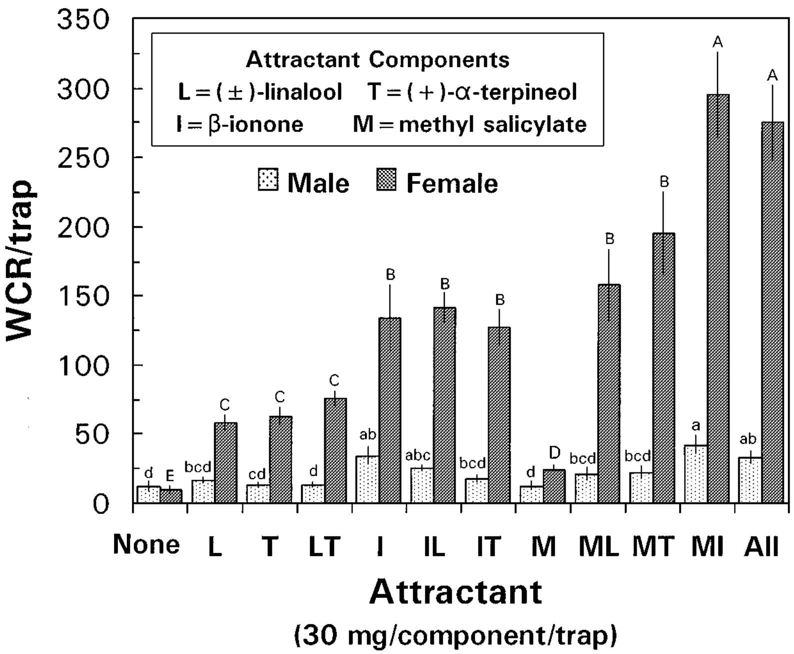

FIG. 2. Effect of blending (±)-linalool, (+)-α-terpineol, β-ionone, and methyl salicylate on WCR capture (mean ± SE, $N = 8$). Means within sexes topped by different letters differ at $P \leq 0.05$ by Student-Newman-Keuls test after ANOVA ($F = 66.62$ and 8.09 for females and males, respectively; $df = 11, 77$; $P < 0.0001$). On September 6, 1996 when the test ended, corn was dough to dent stage (R4–R5) and mean beetle count per plant ± SE was 0.9 ± 0.2 WCR and 1.2 ± 0.2 NCR.

Doses as low as 30 mg/trap (15 mg/chemical) of a 1:1 blend of (±)-linalool and methyl salicylate produced captures higher than those seen with as much as 120 mg of either compound by itself (Figure 3). ROI values calculated for WCR females reacting to the two chemicals, each chemical at doses of 7.5, 15, 30, or 60 mg dispensed from the same and separate traps, were very similar across doses, at 1.9 ± 0.2, 1.8 ± 0.3, 2.1 ± 0.5, or 1.9 ± 0.3 (mean ± SE), respectively. All four doses were, therefore, used to calculate a joint ROI of 1.9 ± 0.2, which differed from 1 ($S = 244$, $N = 32$, $P < 0.001$). Only the higher doses of the blend (60 and 120 mg) attracted NCR females, but the best mean capture (6.4 per trap) did not differ from that to any single component (data not shown). No odorant treatment attracted males of either species (data not shown).

The present tests clearly demonstrated a synergistic increase in capture of WCR females when methyl salicylate and linalool were dispensed from the same as compared with different traps, with observed captures at least 1.9 times those

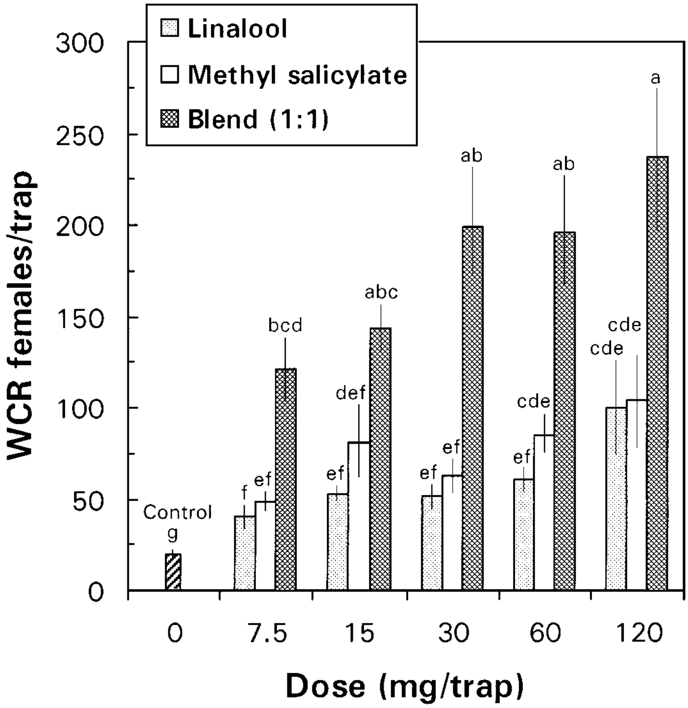

FIG. 3. Relationship between attractant dose and capture (mean $\pm$ SE, $N = 8$) of WCR females for ($\pm$)-linalool, methyl salicylate, and a 1:1 by weight blend of both. Means topped by different letters differ at $P \leq 0.05$ by Student-Newman-Keuls test after ANOVA ($F = 18.50$; $df = 15, 105$; $P < 0.0001$). On August 29, 1997, when the test ended, corn was dough stage (R4) and mean beetle count per plant $\pm$ SE was 1.4 ± 0.2 WCR and 0.9 ± 0.2 NCR.

expected had there been no synergy. The dose–response data showed that a rise of this magnitude would only be expected after about a 10-fold increase in dose of the individual compounds, assuming similar action of both odorants on the same segments of the field population.

Adding β-caryophyllene or indole, but not ($-$)-α-pinene, to a blend of ($\pm$)-linalool and methyl salicylate increased capture of WCR females above levels expected from simply increasing the dose of either the single compound or the binary blend (Figure 4). ROI values calculated in each series from captures on traps baited with 20 mg per component per trap were 2.5 ± 0.7, 1.1 ± 0.1, and 1.2 ± 0.3 for β-caryophyllene, indole, and ($-$)-α-pinene, respectively. Only the β-caryophyllene value differed significantly from 1 ($t = 2.36, N = 8, P = 0.05$; for ($-$)-$\alpha$-pinene, $t = 0.48, N = 8, P = 0.65$; for indole, $S = 5, N = 8, P = 0.55$).

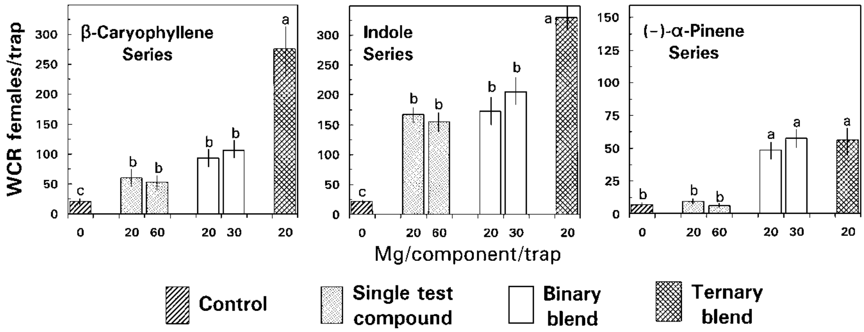

FIG. 4. Mean capture ± SE ($N = 8$) of WCR females with and without addition of β-caryophyllene, indole, or (−)-α-pinene to a 1:1 by weight blend of (±)-linalool and methyl salicylate (20 mg/component/trap for each ternary blend). Means in a series topped by different letters differ at $P \leq 0.05$ by Student-Newman-Keuls test after ANOVA ($F = 17.12$, 98.83, and 36.91, respectively; $df = 5, 35$; $P < 0.0001$). On August 28, 1997, and August 19, 1998, when the β-caryophyllene and indole series ended, respectively, corn was dough stage (R4) and mean beetle count per plant ± SE was 2.3 ± 0.3 WCR and 1.8 ± 0.2 NCR (1997) and 2.8 ± 0.4 WCR and 1.8 ± 0.3 NCR (1998). At the start of the (−)-α-pinene series on August 26, 1999, corn was dent stage (R5) and beetle counts were 1.3 ± 0.2 WCR and 1.0 ± 0.2 NCR.

Odorants lured WCR males just in the indole series, but responses were barely detectable and did not vary among olfactory treatments (data not shown). NCR reacted only to scents containing (±)-linalool, albeit in low numbers, and did not distinguish between binary and ternary blends (data not shown).

Thus, β-caryophyllene, which was only weakly attractive by itself, but not (−)-α-pinene, synergistically elevated female WCR captures when added to the linalool-methyl salicylate blend. Captures were again at least twice as high as expected without synergy. Indole addition to the binary blend produced a WCR response increase that was smaller than the three- to fivefold increase reported for its mixture with 4-methoxycinnamaldehyde or cucurbit volatiles (Metcalf et al., 1995). Indeed, the smaller increase could be explained without invoking synergy if indole and the binary blend were to attract different segments of the WCR female population. For example, one lure could attract females with immature ovaries seeking food and the other affect gravid females seeking oviposition sites. This hypothetical situation is perhaps less likely for WCR, which tend to oviposit where they feed, than for NCR, which feed on a variety of flowering forbs once maize silks dry and then return to maize to oviposit (Branson and Kryson, 1981).

Even 1.0 mg of β-caryophyllene added to 10 mg each of (±)-linalool and methyl salicylate increased captures of WCR females and yielded responses that were not lower than those obtained with additions up to 100 mg (Figure 5). WCR

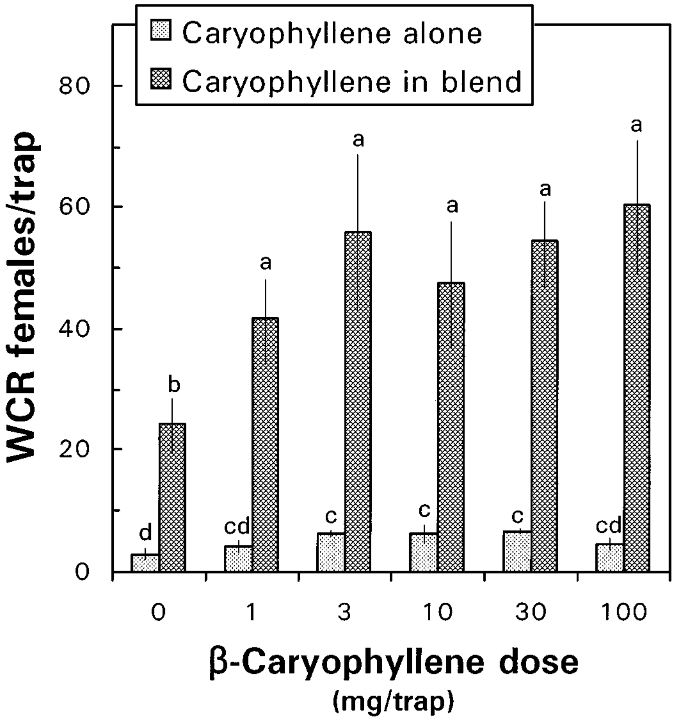

FIG. 5. Relationship between β-caryophyllene dose and capture (mean $\pm$ SE, $N = 8$) of WCR females upon caryophyllene addition to an attractant blend consisting of 10 mg each of ($\pm$)-linalool and methyl salicylate. Means topped by different letters differ at $P \leq 0.05$ by Student-Newman-Keuls test after a significant ANOVA ($F = 41.31$; $df = 11, 77$; $P < 0.0001$). On August 20, 1998, when the test ended, corn was dough to dent stage (R4–R5) and mean beetle count per plant $\pm$ SE was 1.8 ± 0.2 WCR and 3.1 ± 0.4 NCR.

males and NCR of both sexes failed to respond to any odorant treatment in numbers that were different from control (data not shown). Metcalf et al. (1995) also reported only a very gradual linear increase in efficacy as the dose of one member of a binary blend of floral volatiles attractive to WCR was increased over three orders of magnitude (varying dose of indole dispensed with 4-methoxycinnamaldehyde). Stronger dependence on attractant ratios might have been predicted (Loughrin et al., 1996) given the relatively specialized feeding habits of WCR adults (Krysan, 1993; Metcalf and Lampman, 1998) and the ubiquity of many WCR lures. A wider range of component test ratios and quantitative data on component release rates will ultimately be needed to critically evaluate component ratio effects on blend attractiveness.

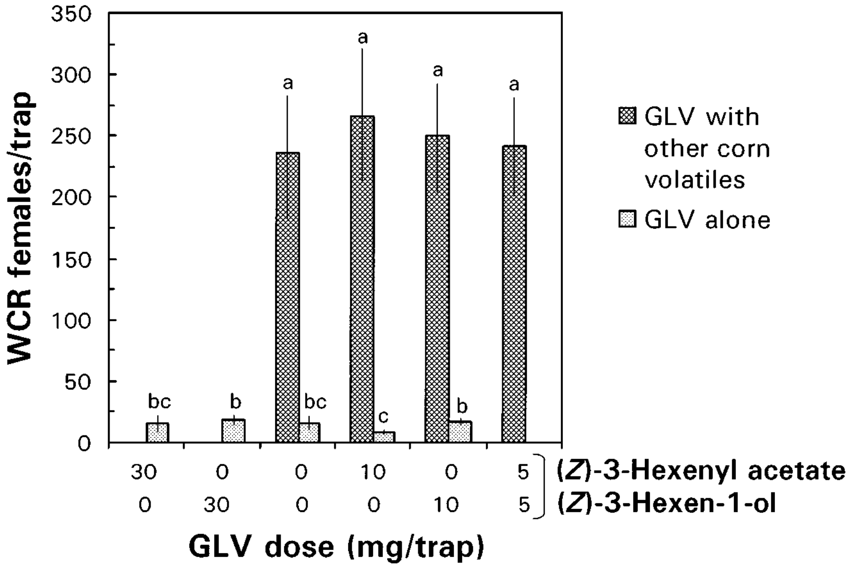

FIG. 6. Effect of the corn green leaf volatiles (GLVs), (*Z*)-3-hexenyl acetate and (*Z*)-3-hexen-1-ol, on WCR female captures (mean $\pm$ SE, $N = 8$) when the volatiles were dispensed singly and blended with other corn volatiles [3.3 mg/trap each of ($\pm$)-linalool, methyl salicylate, and β-caryophyllene]. Means topped by different letters differ at $P \leq 0.05$ by Student-Newman-Keuls test after a significant ANOVA ($F = 82.77$; $df = 8, 71$; $P < 0.0001$). On August 20, 1998, when the test ended, corn was dough to dent stage (R4–R5) and mean beetle count per plant $\pm$ SE was 3.6 ± 0.4 WCR and 1.8 ± 0.2 NCR.

Green Leaf Volatiles. Neither (*Z*)-3-hexenyl acetate nor (*Z*)-3-hexen-1-ol was attractive to WCR females when tested at 10 or 30 mg/trap (Figure 6), despite abundance of these GLVs in the headspace of maize leaves during plant tasseling and silking, when feeding conditions are optimal for corn rootworm adults (Krysan, 1993; Light et al., 1993). Moreover 10 mg of either volatile or a 1:1 (w/w) blend of both also did not affect captures when added to 3.3 mg each of ($\pm$)-linalool, methyl salicylate, and β-caryophyllene (Figure 6). Although GLVs attracting some insects can become inhibitory at higher doses, addition of the acetate and alcohol to the attractive blend of maize volatiles without effect argues against any such inhibition in the present tests. No olfactory treatment in this test significantly affected capture of WCR males or either NCR sex (data not shown).

Blend Efficacy/Enhancement. A blend of ($\pm$)-linalool, methyl salicylate, and β-caryophyllene (each at 3.3 mg/trap) captured fewer WCR of both sexes than did 10 mg of the 4-methoxycinnamaldehyde reference; however, substituting β-ionone for ($\pm$)-linalool yielded a ternary blend that was more attractive than was the

TABLE 3. CAPTURE OF CORN ROOTWORM ADULTS ON TRAPS BAITED WITH (±)-LINALOOL OR β-IONONE BLENDED WITH METHYL SALICYLATE AND β-CARYOPHYLLENE, COMPARED WITH 4-METHOXYCINNAMALDEHYDE STANDARDS[a]

		Capture (mean ± SE)			
		Western corn rootworm		Northern corn rootworm	
Attractant	Dose (mg/trap)	Female	Male	Female	Male
(±)-Linalool blend[b]	10	159.0 ± 27.7c	52.1 ± 10.0c	3.0 ± 1.1	12.0 ± 1.7
4-Methoxycinnamaldehyde	10	306.1 ± 46.9b	102.8 ± 25.3b	1.1 ± 0.4	10.1 ± 1.3
β-Ionone blend[b]	10	542.3 ± 50.1a	138.9 ± 24.7ab	0.6 ± 0.4	14.3 ± 1.5
β-Ionone blend[b]	10				
+ 4-methoxycinnamaldehyde	10	664.9 ± 48.2a	188.6 ± 42.0a	1.5 ± 0.5	7.9 ± 1.7
None (control)	0	18.8 ± 4.5d	26.1 ± 6.5d	2.6 ± 0.8	12.8 ± 2.3
F statistic[c]		203.26**	32.79**	2.40 NS	2.30 NS

[a] Test conducted August 12–14, 1998 ($N = 8$). Mean beetle count per corn plant ± SE on August 14 was 3.7 ± 0.5 WCR and 1.4 ± 0.2 NCR. Corn was in the milk to dough stage (R3–R4).

[b] (±)-Linalool or β-ionone (3.3 mg) blended with 3.3 mg each of methyl salicylate and β-caryophyllene for a total of 10 mg/trap.

[c] Asterisks denote statistical significance at $P < 0.0001$, NS denotes $P > 0.05$ ($df = 4, 28$). Means within a column followed by the same letter do not differ by Student-Neuman-Keuls test, $P > 0.05$.

aldehyde (Table 3). Addition of the aldehyde to the ternary blend containing β-ionone did not affect captures of either WCR sex. No odorant influenced NCR (Table 3).

SUMMARY AND CONCLUSIONS

This study identified two new attractants for corn rootworm adults: *syn*-benzaldoxime and β-caryophyllene. The oxime was notable for its strong attractiveness to NCR, whereas β-caryophyllene synergistically elevated captures of WCR females when blended with other maize headspace volatiles. Methyl salicylate showed a similar synergy when dispensed with one of several maize terpenoids. Indole also increased WCR captures in a manner suggesting synergy but to a lesser extent than reported for its interaction with cucurbit volatiles and their analogs. The results support the conclusion that olfactory synergism plays a key role in host finding by diabroticite corn rootworm adults, in agreement with studies of cucurbit-diabroticite interactions (Metcalf and Lampman, 1997), and demonstrate that the blending of maize volatiles has the potential to dramatically improve efficacy of corn rootworm lures with applications in pest management. Attaining full potential will likely entail adjustment of compound release rates to match natural conditions optimal for corn rootworm attraction, conditions not yet fully elucidated. Most chemical studies of maize volatiles have examined younger crop developmental stages than those preferred by corn rootworm adults or else used cut or macerated tissues, which may release qualitatively and quantitatively different blends than do intact or herbivore-damaged tissues (Buttery and Ling, 1984; Turlings et al., 1993; Takabayashi et al., 1995).

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